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EXPERIMENTAL STUDIES ON THE ETIOLOGY OF CANCER TYPES SPECIFIC TO INDIA

a) Oral Cancer; (b) Kangri Cancer

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Sufficient epidemiological evidence has now accumulated to testify importance of environment — particularly habits and usages in the etiology of human cancer; interesting examples may be cited as chewing tobacco long associated with cancer of the mouth (1, 2, 3, 4), and use of «Kangri» (basket of live coal with Chinar leaves) associated with abdominal skin cancer (5, 6). Factors involved in this etiological hypothesis of two cancer types peculiar to India are investigated by biological testing of tobacco leaf extracts and chinar tar.

(a) Oral Cancer and Testing of Tobacco

Earlier efforts to fractionate sun-cured tobacco and isolate the pure carcinogenic principle from complex tobacco leaves did not meet with much success. Two of the seven extracts of tobacco - an alkaloid containing water extract and total extract of tobacco (designated «E₁») prepared by pooling five extracts together, however, had induced massive hyperplasia of epidermis though animals died within three months of treatment, perhaps due to alkaloid toxicity (7). It was then proposed to investigate fully biological activity of alkaloid-free «total extract» of tobacco in acetone on a sturdy strain of mouse with longer life-span.

EXPERIMENTS AND TECHNIQUES

«Vaddakan» tobacco of Meenampalayam variety was powdered and treated with 2% TICl for removal of alkaloids and then extracted with acetone, obtaining extracts partially free of alkaloids (E₂) and completely free of alkaloids (E₃).

Inbred Swiss and hybrid (Paris albino XVII x C₅₇ Black) mice were used for testing

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carcinogenicity of extracts E₂ and E₃ by cutaneous applications on the interscapular region and by multiple subcutaneous injections. Biweekly skin applications of the extracts were followed by weekly painting of a co-carcinogen Croton-oil. Injected groups received 0.1 c.c. of 2% solution of the extract once a month. The following is the series of experiments conducted:

- I. Cutaneous application of E₂ and E₃ with croton-oil.
- II. (a) Cutaneous application of E₂ with croton-oil,
(b) Multiple subcutaneous injections of E₂.
- III. (a) Cutaneous application of E₃ with croton-oil,
(b) Multiple subcutaneous injections of E₃.
- IV. Cutaneous application of acetone and croton-oil.
- V. Testing of co-carcinogenic activity of tobacco extract with a micro-dose of 3:4-benzpyrene.
 - (a) Single painting of 3:4-benzpyrene, followed by bi-weekly paintings of tobacco extract,
 - (b) Single painting of 3:4-benzpyrene (controls).

Swiss albino and its hairless mutant Swiss (Baldy) mice were used for this experiment.

RESULTS

Detail observations on experimental series I to IV are presented in Table I and summarised in Table II. Table III gives date on the experimental series V. The Tables are self explanatory.

TABLE I
Observations on experimental series I to IV

Experimental Series No.	Type of treatment	Strain of mice (Total N° of animals)	Period of treatment in weeks (N° of animals)	N° of animals with epidermal hyperplasia			Type of tumours		
				Slight	Moderate	Massive	Papilloma of doubtful malignancy	Squamous epidermoid carcinoma	Probable basal cell carcinoma
I	E _a , E _b and Croton-oil painted group	Hybrid (22)	41-60 (1) (+1) 61-80 (12) 81-95 (8)	—	—	1	—	—	—
			3 1	1	3	2	2	3 1	2
		Swiss (11)	41-60 (4) (+2) 61-80 (2) 81-95 (5)	1	1	2	—	—	—
			1 2	— 1	—	—	—	—	—
II(a)	E _a and Croton-oil painted group	Hybrid (28)	41-60 (4) (+3) 61-80 (15) 81-95 (6)	4	—	—	—	—	—
			2 —	3 2	3	1	1*+1 1*+1	—	1
		Swiss (11)	41-60 (2) (+7) 61-80 (2)	1	1	—	—	—	—
			— —	1	1	—	—	—	—
II(b)	E _a Multiple subcutaneous injections	Hybrid (17)	41-91 (17)	—	8	—	—	1	—
III(a)	E _a and Croton-oil painted group	Hybrid (36)	41-60 (4) (+1) 61-80 (8) 81-95 (23)	1 1 2	1 2 4	1 1 1	1* — 2*+4	— — 1+1** 6+1***	— — 1
			— — —	— 1 1	2 1 1	2 1 1	— 1 —	— — —	— — —
		Swiss (18)	41-60 (4) (+8) 61-80 (4) 81-95 (2)	— — — —	2 1 1 1	2 1 1 1	— — — —	— — — —	— — — —
			— — — —	— — — —	— — — —	— — — —	— — — —	— — — —	— — — —
III(b)	Multiple Subcutaneous injections of E _a	Hybrid (17)	41-95 (17)	—	10	—	—	—	—
IV	Acetone and Croton-oil (Controls)	Hybrid (20)	41-60 (6) (+1) 61-80 (1) 81-95 (12)	2 1 4	— — 2	1 — 2	— — 2*+1	— — —	— — —

(+) Animals died or killed before receiving 41 weeks treatment.

* Squamous papilloma
** Carcinosarcoma
*** Carcinoma *in situ*.

E_a - Total Extract of Tobacco.

E_b - Total Extract of Tobacco, partially free of alkaloids.

E_a - Total Extract of Tobacco, totally free of alkaloids.

TABLE II
Summary of observations (experimental Series I to IV) Effect of tobacco extract with a co-carcinogen

Strain of mice	Type of treatment (Painting)	Total No. of animals received more than 40 weeks treatment	Average latent period of papilloma (weeks)	No. of gross papilloma (%)	Papilloma of doubtful malignancy	No. of frank carcinomas (%)
Hybrids of C ₅₇ Black	E _a , E _b & Croton-oil	21	59.5	10 (47.6 %)	2	6 (28.6 %)
	E _b and Croton-oil	25	82.7	9 (43.0 %)	2	2 (8.0 %)
	E ₁₀ and Croton-oil	35	58.1	22 (63.0 %)	4	10 (28.6 %)
	Acetone & Croton-oil (Solvent control)	19	74.3	3 (15.8 %)	1	—
Inbred Strain Swiss	E _a , E _b & Croton-oil	9	57.0	2 (22.2 %)	—	—
	E _b and Croton-oil	4	53.0	2 (50.0 %)	—	—
	E ₁₀ and Croton-oil	10	46.6	3 (30.0 %)	1	—

TABLE III
Effect of tobacco extract as a co-carcinogen (experimental series V)

Type of treatment	Strain of mice (Total No. of animals)	Period of treatment in weeks (No. of animals)	No. of animals with Epidermal hyperplasia			Type of Tumours		
			Slight	Moderate	Massive	Papilloma of doubtful malignancy	Squamous epidermoid carcinoma	Probable basal cell carcinoma
Single painting of 3-4 Benzpyrene and biweekly E ₁₀	Swiss (16)	41-80 (6) 81 onwards (10)	1	—	—	1*	—	—
	Swiss (Baldy) (13)	1-41 (3) 41-80 (10)	2	1	1	1*	1	1
Single painting of 3-4 Benzpyrene (Controls)	Swiss (7)	41-80 (5) 81 onwards (2)	—	—	—	—	—	—
	Swiss (Baldy) (10)	1-40 (6) 41-80 (4)	2	2	1	—	—	—

* Squamous papillomas.

COMMENTS

After years of experimentation with the tobacco extract in varied solvents (7), these are the first series of experiments on tobacco that have yielded frank epidermal carcinomas on mouse skin. The results indicate positive, though weak carcinogenic effect of the tobacco extract that can be demonstrated with the help of a co-carcinogen, croton-oil on hybrid mouse skin. Forty-one of the S1 hybrids showed papillomas (30%), 13 of which (43.9%) developed further into frank carcinomas, the majority being squamous epidermoid type (Fig. 1), the rest indicating a basal cell origin. Nine of the remaining papillomas were doubtfully malignant. All these tumours appeared exclusively in the hybrid mice after 61-95 weeks of treatment. The control group treated with solvent acetone and croton-oil did not develop any carcinoma. The results thus illustrate a weak carcinogenic effect of the tobacco extract.



Fig. 1.

A section of a tumour of a hybrid of XVII & C₃ (Black) developed after 86 weeks of painting of E₁ and croton-oil showing squamous epidermoid carcinoma (Experiment III). x 120.

The acetone extract of tobacco tested as a co-carcinogen in association with 3:4-benzpyrene induced two frank carcinomas and one doubtfully malignant papilloma in 13 Swiss (Baldy) mice (Fig. 2). None of the 10 controls treated only with 3:4-benzpyrene developed tumours. Thus tobacco is capable of promoting the cancerous process initiated by other carcinogenic factor.



Fig. 2.

A palpable tumour in Swiss (Baldy) mouse developed after 14 weeks of cutaneous application of E₁ as carcinogen with one painting of 3:4-benzpyrene (Experiment V).

Muir and Knick's (8) paper reviews extensively the literature on the subject and discusses the role of betel and lime as co-carcinogens in oral carcinogenesis, induced with tobacco. Their data on tobacco carcinogenesis is however, incomplete. As regards co-carcinogenic effect of other ingredients in betel quid, large scale statistical surveys in India have indicated more protective than harmful effect of betel-leaf in tobacco chewers (9). No consideration has therefore been given to these ingredients as co-carcinogens in our studies. Instead factors like local injury, diet deficiencies, spices in diet etc., are being tested as co-carcinogenic factors.

(b) Kangri Cancer.

This is a cancer of abdominal skin in «Kangri users» of Kashmir (5). It was first suggested that the continuous heat of Kangri was a contributory factor responsible for the disease (8). Later the Chinar soot to which the skin is continuously exposed was thought to be the main etiological factor (5). Therefore carcinogenicity of «Chinar tar» is tested by painting the mouse skin. The first series under report includes only Swiss mice — given biweekly paintings with chinar-tar and croton-oil once a week. Table IV presents the observations.

TABLE IV

Effect of 10% Chinar tar*** in benzene and 3% croton-oil in liquid paraffin on skin of Swiss mice

No. of animals and sex	Period of treatment (weeks)	No. of animals with epidermal hyperplasia			No. of animals with papillomas	Average latent period of papillomas	No. of animals with tumours
		Slight	Moderate	Massive			
12	55	2	1**	2+4**	11	40 weeks	2+1* Squamous epidermoid carcinoma
19	60	9	1+1**	1**	8	43.6 weeks	—

* Papilloma of doubtful malignancy

** Squamous papilloma

*** Chinar Tar : prepared by heating dried Chinar leaves at 350°-400°C in a Quick-fit flask and collecting tarry distillate later extracted with benzene.

COMMENTS

Two epidermoid carcinomas and one lesion of



Fig. 3.

A section of the tumour of Swiss female after 55 weeks of skin application of Chinar-tar and croton-oil, showing squamous epidermoid carcinoma.

doubtful malignancy have been observed in 12 female mice (Fig. 3) indicating carcinogenic effect of chinar tar. Eight of the 19 male mice developed papillomas but none of these developed into frank carcinoma. A group of hybrid mice is under treatment. It is also proposed to test heat as a co-carcinogenic factor along with chinar tar simulating «kangri» conditions more closely.

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SUMMARY

RÉSUMÉ

Oral Cancer:

To investigate the role of tobacco in oral cancer, a series of five experiments were carried out to test carcinogenicity of alkaloid-free acetone extract of tobacco. Sixty to 95 weeks' skin paintings of total tobacco extract along with co-carcinogen, croton-oil, induced papillomas in 50% and frank carcinomas in 28.6% of hybrid mice.

The tobacco extract tested as a co-carcinogen with one painting of 3:4-benzopyrene on Swiss (Baldy) mice, also produced tumours.

The data is considered as adequate evidence to prove weak carcinogenicity of chewing tobacco.

Kangri Cancer:

Preliminary testing of «Chinar-tar» in combination with croton-oil induced papillomas in 61.3% and frank carcinomas in 9.7% of Swiss mice — indicating possible carcinogenicity of Chinar-tar.

Cancer oral:

En vue d'étudier le rôle du tabac sur le cancer de la bouche, une série de cinq expériences a été faite pour déterminer le pouvoir cancérogène d'un extrait acétotonique de tabac exempt d'alcaloïdes. Des badigeonnages pendant 60 à 95 semaines à l'extrait total, associés à un co-cancérogène, l'huile de croton, provoquent des papillomes chez 50% et des carcinomes francs dans 28.6% des souris hybrides.

L'extrait de tabac testé en tant que co-cancérogène avec un seul badigeonnage de 3,4-benzopyrène sur des souris Suisses (chauves) a également produit des tumeurs.

Ces résultats sont considérés comme preuve suffisante du faible effet cancérogène du tabac de chique.

Cancer de Kangri:

Les épreuves préliminaires sur le «Chinar tar» combiné à l'huile de croton ont donné des papillomes chez 61.3% et des carcinomes francs chez 9.7% des souris Suisses indiquant ainsi un pouvoir cancérogène possible du «Chinar tar».